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The Influence of HSV-1 Infection on Circadian Rhythms and Behavior in Mice

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Herpes Simplex Virus-1 Affects Circadian Rhythmicity in Mice

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Background

- As of 2015, WHO indicates that 67% of the population carries herpes simplex virus-1 (HSV-1)
- Viral infection fluctuates based on circadian protein oscillations in terms of BMAL1 and Cry. HSV-1 infection is also associated with oxidative damage in neurons
- Circadian oscillations are responsible for synchronizing vital physiological processes including sleep, body temperature and immune defense. disruption of circadian rhythms can induce neurological and physiological changes that are detrimental to the organism (Edgar et al., 2016)
- There is correlational evidence that suggests that individuals with major mood disorders have significant herpesvirus infection in GABAergic purkinjie neurons. This may be a mechanism leading to the prevalence of these disorders (Prusty et al., 2018)
- It is well understood how the molecular circadian systems are altered in HSV-1 infection, however it is not understood what behavioral neurological changes are observed from latent HSV-1 infection
- In the present study, behavior and circadian rhythms were analyzed in mice inoculated with HSV-1 or saline through corneal scarification and activity patterns were collected through infrared sensors

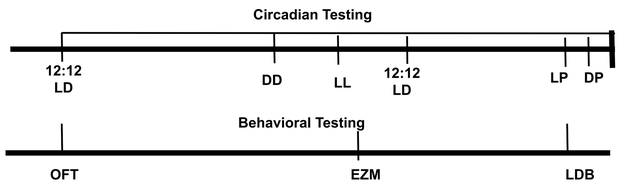
- Mice were tested in the following apparatuses:
  - Open field test (OFT) for locomotion and anxiety-like behaviors
  - Elevated zero maze (EZM) for risk-taking behaviors
  - Light/Dark box (LDB) for anxiety-like behaviors

- We predicted that HSV-1 mice would demonstrate more anxiety-like behaviors than their control counterparts.

- We also predicted that HSV-1 mice would exhibit significant abnormalities in circadian rhythmicity, characterized by changes in circadian period and alpha.

Methods

- 8-week-old male BALB/c mice were inoculated with HSV-1 (n=9) or Saline (n=9) through corneal scarification
- Mice were individually housed in cages with overhead Infrared Motion Detectors. Activity was continuously counted in one-minute intervals.
- Mice were assessed for circadian rhythms:
  - 12:12 light-dark conditions (LD) two-weeks before inoculation
  - 12:12 LD for 3 weeks following inoculation
  - Constant darkness conditions (DD) for 2 weeks
  - Constant light conditions (LL) for 2 weeks
  - Return to 12:12 LD for 2 weeks
  - 2-hour light pulse (LP) administered, from Zeitgeber time (ZT) 14 during 12:12 LD
  - 2-hour dark pulse (DP) administered from ZT 2 during 12:12 LD
- Behavioral tests were performed 10-weeks post-inoculation to assess anxiety-like behaviors:
  - OFT: exploration of center and perimeter of apparatus for 5 minutes
  - EZM: exploration of walled and open areas of apparatus for 5 minutes
  - LDB: exploration of light, dim, and dark areas of apparatus for 5 minutes
- Histology:
  - 20 weeks post-inoculation
  - collected suprachiasmatic nucleus (SCN) and trunk blood
- Analysis:
  - Circadian data: ActogramJ
  - Statistical data: SPSS

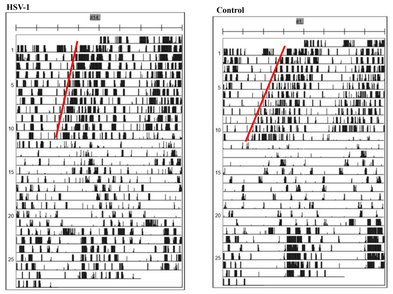


Acute HSV-1 infection decreased activity during the night

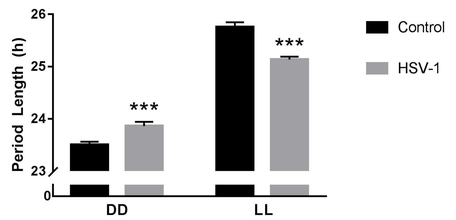
Circadian rhythms are affected by HSV-1 infection

Anxiety is not affected by HSV-1

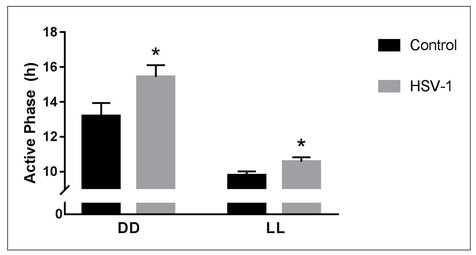
Results



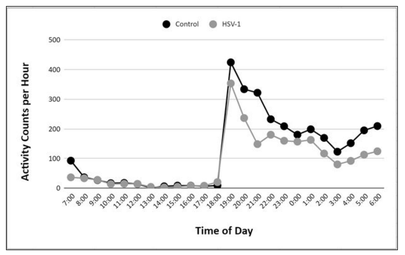
Note: Representative actograms of HSV-1 (left) and Control (right) animals. Activity counts, as visually represented by the tick marks on this graph, were double plotted (each mark in the top segment represents six hours) for LD (days 1-11), DD (days 12-22), and LD (23-27). This actogram highlights the entrained activity patterns during LD, and the circadian rhythms that become visible in constant conditions. LD = light/dark cycle; DD = dark/dark cycle; HSV-1 = Herpes Simplex virus 1.



Note: Average period length, as manually calculated on ActogramJ off of the actogram analyses, for both control and HSV-1 groups were plotted in DD and LL. In DD, the period of the HSV-1 group was significantly longer than controls, $t(16) = -3.638$, $p = 0.002$. In LL, the period of the HSV-1 group was significantly shorter than controls, $t(16) = 5.954$, $p < 0.001$. HSV-1 = Herpes Simplex virus 1; DD = dark/dark cycle; LL = light/light cycle. Significance is defined as $\alpha = 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.



Note: Average alpha for both control and HSV-1 groups were analyzed in DD and LL based on the actogram analyses. In DD, the alpha of the HSV-1 group was significantly longer than controls, $t(16) = -2.231$, $p = 0.040$. In LL, the alpha of the HSV-1 group was significantly longer than controls, $t(16) = -2.325$, $p = 0.034$. HSV-1 = Herpes Simplex virus 1; DD = dark/dark cycle; LL = light/light cycle; alpha = length of active phase. Significance is defined as $\alpha = 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.



Note: Average activity count profiles prior to inoculation for both the control and HSV-1 groups are presented, n=15; HSV-1 = 8, Control = 7. HSV-1 activity levels were significantly lower than the controls in the 7-days post-inoculation time frame $t(13) = 3.108$, $p = 0.008$. LD = light/dark cycle; sham = control group; HSV-1 = Herpes Simplex virus 1. Significance is defined as $\alpha = 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

Discussion

- HSV-1 inoculation decreased activity during the night
  - BALB/c mice are nocturnal, meaning they are similarly less active during the non-active phase, but also significantly less active while they should be during their active phase

- HSV-1 inoculation affected altered multiple circadian rhythm parameters
  - Period - significant increase in DD, but significant decrease in LL
  - Alpha - significantly larger in both DD and LL

- These disruptions in circadian rhythms are consistent with the literature
  - Correlations between other viruses and circadian rhythms (Zhuang et al., 2012)
  - HSV-1 and disruption of CLOCK mechanisms (Zhuang et al., 2012)
  - HSV-1 is associated with chronic fatigue syndrome (Bond & Dinan, 2006)

- There were no changes in anxiety-like behaviors
  - Consistent with lack of support for correlations with psychiatric disorders
  - Further testing needed to support this finding

- Our research **supports** our hypothesis that HSV-1 inoculation affects the circadian rhythms of BALB/c mice, but does **not support** our hypothesis that HSV-1 infection will increase anxiety-like behavior

Conclusions

- HSV-1 inoculation significantly altered multiple circadian rhythm parameters
- Circadian abnormalities were not due to changes in anxiety-like behaviors by HSV-1 treatment

Future Work

- Analyze female circadian patterns
- Use brain samples to analyze abnormalities caused by the HSV-1 treatment on the SCN and trunk blood

References

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